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A Novel Simple Method for Assessing Rodent Innate Fear Using Round Elevated Platform

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Introduction

Open field and elevated plus maze have been widely used for assessing rodent innate fear. In OF task, animals with more fear will spend less time in the center zone. Regarding EPM, animals with more fear will spent less time in the open arms. Thus less activity in unsafe zone will be observed in animals with more fear. Based on this assumption, we speculated that our previously designed opaque round elevated platform (O-REP) with open space can be used for assessing rodent innate fear by analyzing animal activity. In O-REP, height and the open space make the animals feel fearful in O-REP and decrease its activity. To verify this novel method, the difference between female and male mice were examined by OF, EPM, and O-REP respectively. Fear encompasses both learned fear and innate fear, which is normal emotion with great adaptive value that has been selected along the evolutionary process . Though both learned and innate fear responses are controlled by the amygdala complex, they are definitely different. Learned (Acquired) fear triggers characteristic behaviors of escape and avoidance in response to a specific, previously experienced stimulus, such as pain or the threat of pain. In contrast, innate fear is genetically encoded and does not require response learning . Our understanding of learned fear is largely based on studies of Pavlovian fear conditioning, in which an initially neutral conditioned stimulus (CS) of any sensory modality (such as sound) is paired with an innately aversive unconditioned stimulus (Such as electric foot shock). To test learned fear level, freezing time after CS was used as index for characterizing fear level . Different from learned fear, entries into and time spent in the unsafe environment was usually to assess the animal innate fear. Based on this assumption, open field (OF), light-dark box (LDB), elevated plus maze (EPM), Social interaction test was usually used to examine the rodent innate fear . All of the above tasks can simultaneously provide a relative safe (familiar) area and an unsafe (unfamiliar) environment, which allow the animals to freely approach the novel area (open space, height and bright lit) to satisfy its curiosity, while avoid it when feeling afraid. Thus the psychological conflict between

exploring drive and motive to avoid aversive unsafe environment was used to reflect animal anxiety, and the exploring time in unsafe zone was used to reflect innate fear . The more time spent in the unsafe zone, the less fear level will be displayed by the animals. However, due to the different compartment settings, the results from these apparatus are sometimes inconsistent. In addition, for all mammalians, Security requirements was the first requirement should be satisfied. Thus, the safe areas of the mexisting apparatus will decrease the motive to explore in the unsafe zone, which make them not sensitive enough to examine animal innate fear difference in some content. For OF task, walls provide animals a safe area and animals spent most of test duration in this area. They will feel safe in this area and may be not willing to explore in the center zone, other than afraid to explore. In the EPM, close arms provide a dark and safe compartment, while the open arms provide an unsafe zone. According to its principle, the more time spent in the open arms, the less anxiety and fear will be displayed by the animals. However, rodent prefer staying in the dark environment, thus sometimes, animals doesn't travel to open arms may be due to their preference in dark zone, but not only due to its fear. In our experiments, some mice even stay in the closed arm during the all test duration and never enter into the open arms. For the light-dark box, it comprises a light box (aversive area) and a dark box. Similar with EPM, animal prefer staying in dark environment, thus less entries into or time spent in the light box doesn't necessarily mean that they feel fearful. In Social interaction test, familiar mouse may satisfy its social requirement and no need to approach the unfamiliar mouse . Thus these limitations greatly affect their effectiveness in assessing animal fear. To overcome these limitations, we use an Opaque Round Elevated Platform (O-REP, 40cm above the floor) with open space to evaluate rodent innate fear, which has been used for examining rodent anxiety previously . Because there is no walls and enclosed compartment was designed in this apparatus, so the mice on the O-REP will have no safe zone to hide and feel rather afraid, which will results in decrease of activity (travel less distance). Thus the travelling distance in O-REP may be used for reflecting rodent innate fear. The outer zone of O-REP is similar with the open arm of EPM, and the open space is similar with the center zone of OF apparatus, all of which were utilized to produce fearful challenge . Thus the fearful challenge in O-REP should be higher than that in OF and EPM. As we know, dangerous environment is easier than safe environment to discriminate brave animals and less brave animals. Therefore, the O-REP may be more sensitive in finding innate fear difference between different groups. Due to the different fear level between female and male, to test our O-REP task, innate fear difference between female and male mice was examined by EPM, OF and O-REP, and the data from these three tasks was compared. In addition, the effect of shape and transparency of the EP was also investigated, in hope that we can

provide researchers a novel sensitive method for assessing rodent innate fear.

Fear encompasses innate fear and learned fear, both of which play pivotal role in surviving animals. Learned fear triggers

characteristic behaviors of escape and avoidance in response to previously experienced stimulus. In contrast, innate fear triggers the escape behavior in response to the unsafe environment or the predators. Thus the methods for assessing these two kinds of fear are different. Present methods for examining learned fear were based on pavlovian fear conditioning, and lots of the detailed protocol of which has been reported . Methods for assessing innate fear was usually investigated by examining the animal response to potential threat or fearful environment, such as the height, the open space, bright lit, looming shadows, smell of predators, auditory threat cues. OF and EPM task were based on this and have been widely used for assessing rodent innate fear. OF task provide an open space in the center, and rodent feel afraid in this area, thus the time spent in this zone can reflect the rodent innate fear. Animals with less innate fear will spend more time in the center zone of OF. Regarding EPM, open arms provide the height to make the animal feel afraid, and animals with less innate fear will spent more time in the open arms. However, these two methods are not sensitive enough and the results from the two methods are sometimes inconsistent, which limit the innate fear research. Previously, we designed a novel elevated platform (O-REP) for assessing rodent anxiety and locomotor activity . Recently, we found that mouse travelled remarkably less distance in this O-REP task than that in OF task, which indicated that more fearful challenge was produced in O-REP compared with that in OF, thus urge us to discuss the feasibility of examining the rodent innate fear and anxiety by analyzing the intensity of animal activity in O-REP, and the animals with higher innate fear level will be afraid to move, thus should travel less distance. To test our speculation, the innate fear difference between female and male mice was examined by EPM, OF and O-REP task respectively. As shown in, EPM was unable to find the fear difference between female and male mice, including C57/BL6 and ICR mice. However, OF task did find that C57/BL6 female mice displayed less innate fear than male mice, which is inconsistent with the results from EPM task. Excitedly we found that O-REP task also found the significant difference between C57/BL6 female and male mice, which indicated that O-REP task might be feasible to assess rodent innate fear. In contrast, OF task was unable to find the fear difference between ICR female and male mice, but O-REP did find significant difference of fear between them, indicating the higher sensitivity of O-REP in assessing innate fear.

The results showed that EPM task could not find the significant fear difference between female and male mice using ICR and C57/BL6 strain. However, C57/BL6 female mice displayed less fear both in OF and O-REP task, which indicates that OF and O-REP task displayed more sensitivity than EPM task in assessing rodent innate fear. Furthermore, O-REP task rather than OF task found that female ICR mice displayed significantly more innate fear in O-REP, which indicated that O-REP might be more sensitive than OF and EPM in assessing innate fear. Further investigations showed that the animals displayed similar activities in O-REP, opaque square elevated platform (O-SEP) and transparent round elevated platform (T-REP), indicating that shape and transparency of EP did not affect the sensitivity of EP. Thus our data demonstrated that the REP is novel alternative task for evaluating rodent innate fear, which sensitivity was not affected by the shape and transparency. The present studies will greatly facilitate fear related research

Keywords: Innate fear; Open field; Elevated plus maze; Round elevated platform